EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400

Title: Inhibition of *Listeria monocytogenes* by Native Microflora of Whole Cantaloupe

Author(s): D.O. Ukuku, W.F. Fett, and G.M. Sapers

Citation: Journal of Food Safety (2004) 24: 129-146

Number: 7485

INHIBITION OF *LISTERIA MONOCYTOGENES* BY NATIVE MICROFLORA OF WHOLE CANTALOUPE[1]

DIKE O. UKUKU[2], WILLIAM F. FETT and GERALD M. SAPERS

Food Safety Intervention Technologies Research Unit
U.S. Department of Agriculture
Agricultural Research Service
Eastern Regional Research Center
600 East Mermaid Lane
Wyndmoor, Pennsylvania 19038

Accepted for Publication February 23, 2004

ABSTRACT

Fresh-cut cantaloupe has been recalled due to the possible presence of Listeria monocytogenes. *Several studies have reported that naturally occurring microflora of vegetable surfaces may be antagonistic to pathogen attachment, growth or survival. To test this possibility for* L. monocytogenes *and cantaloupes, whole melon were treated with water, ethanol (70%) or chlorine (200 ppm) to reduce the native microflora on the melon surfaces. Treated or untreated melons were immersed in a six strain cocktail of* L. monocytogenes *(10^7 CFU/mL) for 10 min and then allowed to dry for 1 h inside a biosafety cabinet followed by storage at 5, 10 and 20C for 15 days. Fresh-cut pieces prepared from the treated or untreated melons and directly inoculated with* L. monocytogenes *(3.48 log CFU/g) were stored under the same conditions listed above. Populations of* L. monocytogenes *and five classes of native microflora were investigated. Growth of* L. monocytogenes *in sterile or nonsterile rind and fresh-cut homogenates was also studied. The population of* L. monocytogenes *recovered from inoculated (10^3 to 10^8 CFU/mL) whole melons given no disinfection treatment or washed with water was significantly less ($P < 0.05$) than that recovered from melons treated with chlorine or EtOH. In general, populations of* L. monocytogenes *declined on the surface of treated and untreated whole melons and on fresh-cut pieces over the 15 days storage period at the temperatures tested. However, the decline in pathogen populations was less rapid in the presence of reduced populations of native microflora. Higher*

[1] Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

[2] Author for correspondence. TEL: 215-233-6427; FAX: 215-233-6406; EMAIL: dukuku@arserrc.gov

populations of L. monocytogenes *were attained in sterile tissue homogenates than in nonsterile homogenates. Addition of yeast and mold to sterile rind homogenates was highly inhibitory to growth and survival of the pathogen. The results of this study indicate that native microflora of whole cantaloupe inhibited attachment to rind surfaces as well as survival and growth of* L. monocytogenes *on cantaloupe surfaces and homogenized fresh-cut pieces. Thus,* L. monocytogenes *recontamination of melons having a reduced level of native microflora following application of a disinfection treatment may be a food safety concern.*

INTRODUCTION

Listeria monocytogenes is a particular food safety concern because it is widespread in the environment (Beuchat and Brackett 1991; Cox *et al.* 1989), grows under refrigeration conditions (Farber and Peterkin 1991; Marston 1995), and is a frequent resident in certain food processing establishments (Carlin and Nguyen-The 1996; Brackett 1988; Heisick *et al.* 1995). The ability of *L. monocytogenes* to attach to a variety of surfaces and its virulence characteristics also contribute to this concern (Mafu *et al.* 1990). The microorganism has been isolated from soil, sewage sludge, vegetation, and water (Brackett 1988; Cox *et al.* 1989) and, therefore, has the potential to contaminate cantaloupe surfaces. Many vegetables, including bean sprouts, cabbage, cucumber, potatoes, and radishes have been found to be contaminated with *L. monocytogenes* (Berrang *et al.* 1989; Beuchat 1996; Cox *et al.* 1989; Farber *et al.* 1989; Heisick *et al.* 1989; Heisick *et al.* 1995). The pathogen has been reported to survive long term storage on leafy vegetables (Francis and O'Beirne 1997; Carlin and Nguyen-The 1994), has been responsible for numerous product recalls of salads (Wong *et al.* 2000) and was responsible for an outbreak of foodborne disease due to coleslaw prepared from contaminated raw cabbage (Beuchat 1996).

Even though there are no documented reports of an outbreak of human listeriosis associated with the consumption of contaminated fresh-cut cantaloupe, the potential for such an outbreak remains a concern as evidenced by a recent recall of fresh-cut honeydew and cantaloupe melons (FDA 2003) and fresh-cut fruit salad due to potential contamination with *L. monocytogenes* (Kroger 2000). Sporadic illnesses have been linked with *L. ivanovii* and one case of meningitis with *L. seeligeri* (Farber and Peterkin 1991), but *L. monocytogenes* remains the major pathogen to man.

There is a potential for the use of native microflora to reduce pathogen growth and survival on fruits and vegetables. Vescovo *et al.* (1996) reported use of lactic acid bacteria to control pathogens in ready-to-use vegetables. Other researchers have reported use of lactic acid bacteria to improve the safety of minimally processed fruits and vegetables (Breidt and Fleming 1997; Leibinger

et al. 1997; Torriani *et al.* 1997). Recently we reported that *Listeria monocytogenes* inoculated on whole cantaloupe surfaces declined during storage at 5 and 20C for 15 days (Ukuku and Fett 2002b). We hypothesized that the native microflora of whole cantaloupe are antagonistic to the survival of the pathogen, which may be responsible for the absence of reported outbreaks of listeriosis associated with consumption of fresh-cut cantaloupe. In this study, we investigated the effect of native microflora of whole cantaloupe on attachment, survival and growth of *L. monocytogenes* on the surfaces of cantaloupe or in homogenates of cantaloupe rind and fresh-cut pieces.

MATERIALS AND METHODS

Source of Melons

Unwaxed cantaloupes (1682 ± 34 g, western shippers) purchased from a local produce warehouse were allowed to come to room temperature (~20C) overnight before being inoculated.

Bacterial Strains and Inoculum Preparation

A mixed bacterial cocktail containing six strains of *L. monocytogenes* [F8027 (celery, Serotype 4b), F8385 (carrot, Serotype 1/2b), and G1091 (coleslaw, Serotype 4b), were received from Dr. Larry Beuchat, Univ. of Georgia; and CCR1-L-G (food isolate), ATCC 15313 (type strain) and H7888 (food isolate) was utilized. Except where designated, bacterial strains were obtained from the USDA-ARS-ERRC culture collection and was prepared by two successive loop transfers of the individual strains at 18 h intervals (37C) in 5 mL Trypticase Soy Broth (TSB, BBL/Difco, Sparks, MD) supplemented with 0.6% yeast extract (BBL/Difco) (TSBY). A final transfer of 0.2 mL was made into 20 mL TSBY with incubation at 36C for 18 h under static conditions. Populations of individual cultures before mixing ranged from 1.38 to 2.19 × 10^9 CFU/mL as determined by plating serial dilutions on Tryptic Soy Agar (TSA, BBL/Difco) with incubation at 37C for 24 h. The bacterial cells were harvested by centrifugation (10,000 *g*, 5 min) at 4C. The cell pellets were washed twice in salt-peptone [0.85% NaCl, 0.05% Bacto-peptone (BBL/Difco)], and the cell pellets were transferred to 3 L of 0.1% peptone water (PW) to make the inoculum cocktail. The final bacterial concentration in the inoculum was 8.58 × 10^7 CFU/mL. When required, the inoculum was diluted with 0.1 % PW to appropriate concentrations of 10^3 to 10^8 CFU/mL.

Preparation of Sanitizing Solutions

Clorox, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Company, Oakland CA), was diluted with sterile water to provide a concentration of 200 ppm of available chlorine in the wash solution. The pH was adjusted to 6.4 ± 0.1 by adding citric acid. Free chlorine in the solution was then determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency. A 70% aqueous ethyl alcohol (EtOH) solution was prepared from 200 proof EtOH (U.S.P., Warner-Graham Co., Cockeysville, MD).

Sanitizing Whole Melons

Whole cantaloupes were divided into three groups: one group was surface treated with 70% EtOH, the second group was treated with 200 ppm chlorine and the third group was washed with water to reduce total microflora. The treatment with water, EtOH and chlorine were accomplished by submerging individual cantaloupes in 3 L of each solution for 3 min with manual rotation and rubbing of the melon surface to assure complete coverage and contact of surfaces with the wash solution. Treated cantaloupes were drained and placed inside a biosafety cabinet for 1 h to dry.

Native Microflora of Cantaloupe Surfaces

Random samples of 70 plugs per cantaloupe weighing approximately 25 g (with a total surface area of 266 cm^2) were blended [Waring commercial blendor (Dynamic Corp, New Hartford, CT), speed set at level 5, for 1 min] with 75 mL of 0.1% PW. Decimal dilutions of the sample were made with 0.1% PW, and aliquots (0.1 mL) were plated in duplicate on a range of media. Plate Count Agar (PCA, BBL/Difco) with incubation at 30C for 3 days was used for enumeration of mesophilic aerobes. *Pseudomonas* spp. were enumerated by plating 0.1 mL on *Pseudomonas* isolation agar (PIA, BBL/Difco) with incubation at 27C for 3 days. Lactic acid bacteria were enumerated with de Man, Rogosa and Sharpe agar (MRS, Oxoid, Ogdensburg, New York) with 0.08% sorbic acid as a supplement with incubation at 30C for 3 days (Reuter 1985). Yeast and mold were enumerated according to Norris and Ribbons (1971) using Czapek Malt Agar (CMA, Sigma, St. Louis, MO). For use in co-cultivation experiments with sterile or non-sterile rind and fresh-cut homogenates, yeast and mold colonies from the CMA plates were scraped into 5 mL Sabouraud Maltose Broth (SMB, BBL/Difco), mixed and then incubated at 25C for 4 days. Classes of native microflora (aerobic mesophiles, *Pseudomonas* spp. and lactic acid bacteria) isolated from PCA, PIA, and MRS plates were individually suspended in 10 mL BHIB with a sterile transfer loop. The samples

were incubated at 27C for *Pseudomonas* spp or 30C for aerobic mesophiles and lactic acid bacteria for 3 days, respectively. A final transfer (0.2 mL) of each class of native microflora was made into 20 mL BHIB with incubation at 30C for 3 days or SMB (yeast and mold only) with incubation (static) at 25C for 4 days under static conditions. Bacterial cells and the yeast and mold were harvested by centrifugation (10,000 *g*, 5 min) at 4C and the cell pellets were washed in salt-peptone [0.85% NaCl, 0.05% Bacto-peptone (BBL/Difco)]. The final cultures of native bacteria and yeast and mold were used to inoculate sterile rind or fresh-cut homogenates as described below.

Inoculation of Whole Melon and Fresh-Cut Pieces

Surface sanitized, water washed and untreated whole melons were inoculated with different levels of *L. monocytogenes* (3.00 to 7.58 $\log_{10}$ CFU/mL) by submerging the melons in 3 L of inoculum and agitating by constant stirring with a glove covered hand for 10 min to ensure even exposure to the inoculum. After inoculation, the cantaloupes were allowed to dry for 1 h in a biosafety cabinet and then stored at 5C or 25C for up to 15 days before sampling.

In a separate experiment fresh-cut pieces, prepared from untreated or sanitized (200 ppm chlorine or 70% EtOH) whole melons were inoculated with 10^3 to 10^6 CFU/mL *L. monocytogenes* by submerging the fresh-cut pieces in 3 L of inoculum and agitating by constant stirring with a glove covered hand for 1 min to ensure even exposure to the inoculum. Inoculated samples were stored in sterile Stomacher™ bags (Dynatech Laboratories, Alexandria, VA) at 10C and were sampled for growth of *Listeria* at day 0, 2, 4, 6, 8, 10, 12 and 15 days as described below.

Preparation and Inoculation of Rind and Fresh-Cut Homogenates

To prepare homogenates, cantaloupe rind samples (50 plugs, ~21 g) from untreated whole melons were blended (Waring commercial blender, speed set at level 5 for 30 s) with 80 mL sterile water and then transferred to a filter Stomacher® bag (Dynatech Laboratories, Alexandria, VA). Similarly, homogenates of fresh-cut pieces from the untreated whole melons were also prepared. Fresh-cut pieces (~ 970 g/whole cantaloupe) were divided into 2 groups of 485 g, homogenized with 500 mL sterile water and filtered as above. One filtrate for each group of samples was autoclaved at 121C for 15 min to kill the native microflora. The sterile filtrates were then poured out into sterile 125 mL Erlenmeyer flasks (40 mL per flask), placed in an ice bath and allowed to cool to ~ 23C. A mixed cocktail (1 mL) of the *L. monocytogenes* strains was inoculated into 40 mL of sterile rind or fresh-cut homogenate filtrate and TSBY (at a concentration of 2.56 log CFU/mL), with incubation at 5, 25, and 36C

under static conditions. In separate experiments, four classes of native microflora (*Pseudomonas*, yeast and mold, lactic acid bacteria and aerobic mesophilic bacteria), isolated from whole cantaloupe surfaces as described above were diluted at 2.43 log CFU/mL for each class of native microflora into the sterile rind or fresh-cut homogenate filtrates. The sterile rind or fresh-cut homogenate filtrates containing each class of these native microflora was inoculated with a cocktail of *L. monocytogenes* at 2.56 log CFU/mL. Viable cell populations in the homogenates were determined at days 0, 3, 6, 9, 12 and 15 by standard dilution plate techniques as described below.

Microbiological Analyses

Cantaloupe rind and fresh-cut pieces were homogenized, serial dilutions were prepared in 0.1% PW, and aliquots (0.1 mL) were plated on different agar media as stated above. In addition, Enterobacteriaceae were determined using a pour plate method on Violet Red Bile Glucose Agar (VRBGA, Oxoid) with an overlay of the same agar after solidification with incubation at 30C for 3 days. For *L. monocytogenes*, *Listeria* identification agar (PALCAM, Sigma) containing *Listeria* selective supplement (L-4660, Sigma) was used with incubation at 37C for 48 h (Lovett and Hitchins 1988). All plating was done in duplicate. Pure cultures of *L. monocytogenes* were surface plated onto PALCAM agar to serve as references for identification. Representative presumptive colonies of *L. monocytogenes* were subjected to confirmation by use of API *Listeria* test kits (bioMeriux Marcy l'Etiole, France).

Statistical Analyses

All experiments were replicated three times. Microbial data were converted to $\log_{10}$ CFU/cm^2 or $\log_{10}$ CFU/g and where appropriate analyzed for differences in response to treatments using analysis of variance (ANOVA) and the Bonferroni LSD (Miller 1981) method to determine significant differences ($P \leq 0.05$). All statistical analyses were performed with Statistical Analysis System (SAS).

RESULTS

Effect of Sanitizers on Native Microflora

The population of native microflora on cantaloupe rinds averaged 6.82 $\log_{10}$ CFU/cm^2 for total mesophilic aerobes, 2.90 $\log_{10}$ CFU/cm^2 for yeast and mold, 4.00 $\log_{10}$ CFU/cm^2 for Enterobacteriaceae, 3.00 $\log_{10}$ CFU/cm^2 for *Pseudomonas* spp. and 3.86 $\log_{10}$ CFU/cm^2 for lactic acid bacteria. Sanitizing whole

cantaloupes with chlorine (200 ppm) or EtOH (70%), caused a significant ($P < 0.05$) reduction of all five classes of naturally occurring microflora on the rind (Fig. 1). Washing with water was ineffective resulting in reductions of less than 0.5 $\log_{10}$ for the class of native microflora. Treatment with chlorine was more effective than EtOH in reducing all classes of native microflora except *Pseudomonas* spp. EtOH and chlorine treatments caused a 1.39 and 2.86 $\log_{10}$ CFU/cm^2 reduction of mesophilic aerobic bacteria (APC), respectively.

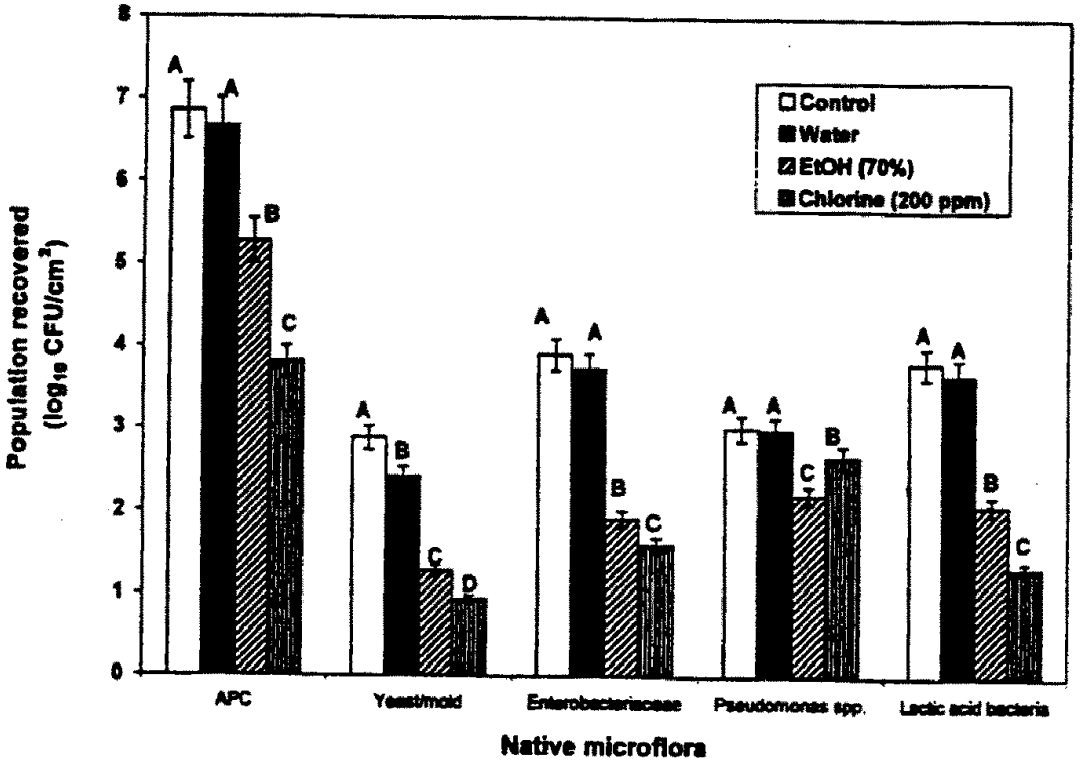

FIG. 1. EFFECT OF SANITIZER TREATMENT ON POPULATIONS OF SPECIFIC CLASSES OF NATIVE MICROFLORA ON WHOLE CANTALOUPE SURFACES

Error bars represent ± standard deviation for each class of native microflora population. Means of data for each individual class of native microflora not followed by the same letter are significantly different ($P < 0.05$).

Populations of native microflora recovered from fresh-cut pieces prepared from treated and untreated whole melons are shown in Fig. 2. Sanitizer treatments of whole melons reduced the transfer of all five classes of native microflora to fresh-cut pieces and, in most instances, the reductions were statistically significant ($P < 0.05$). EtOH and chlorine treatments of the whole melon caused a 0.79 and 1.46 $\log_{10}$ CFU/g reduction of mesophilic aerobes on the pieces, respectively.

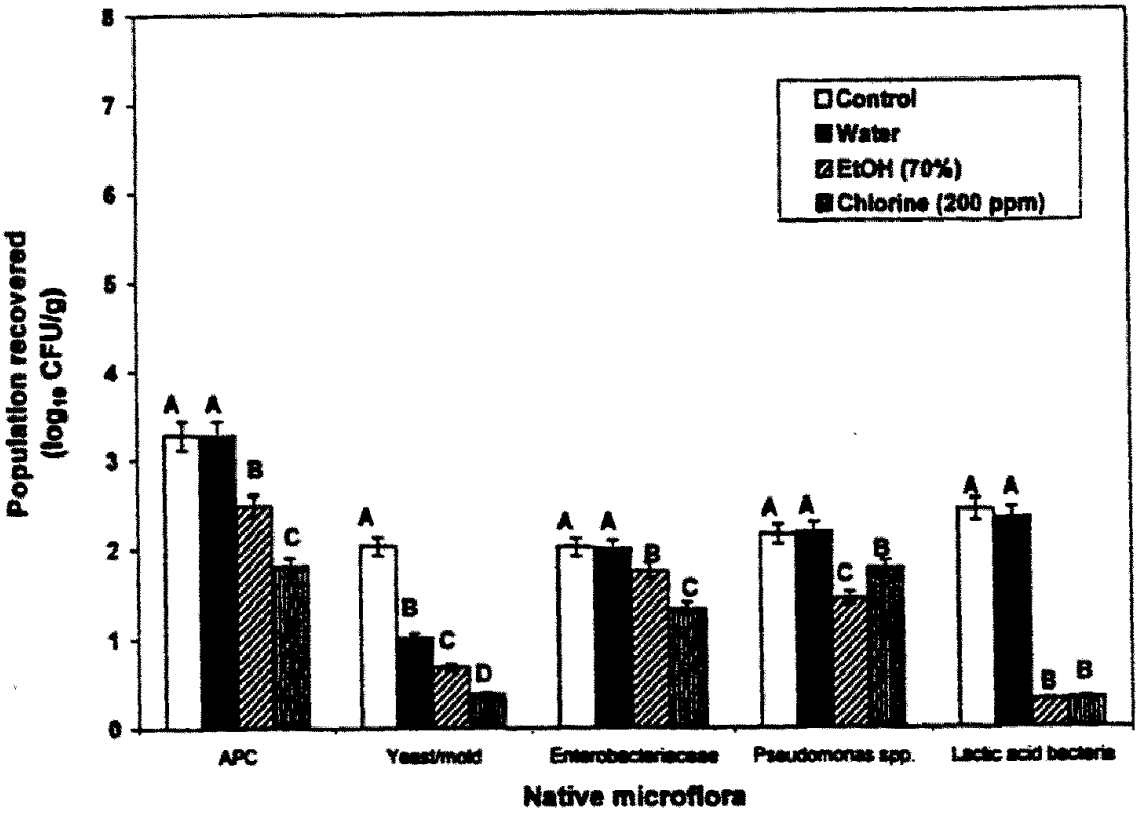

FIG. 2. EFFECT OF SANITIZER TREATMENT ON TRANSFER OF SPECIFIC CLASSES OF NATIVE MICROFLORA FROM WHOLE CANTALOUPE SURFACES TO FRESH-CUT PIECES

Error bars represent ± standard deviation for each class of native microflora population. Means of data within individual classes of native microflora not followed by the same letter are significantly different ($P<0.05$).

Attachment of *L. monocytogenes* on the Surface of Whole Melon

Listeria monocytogenes was not detected from the surface of uninoculated whole cantaloupe. The attachment of *L. monocytogenes* on the surface of untreated whole melons or melons treated with water, chlorine (200 ppm) or 70% EtOH, as influenced by inoculum density, is shown in Fig. 3. The highest populations of *L. monocytogenes* recovered were after inoculation with *L. monocytogenes* at the highest inoculum level tested (7 log CFU/mL) irrespective of surface treatment. Attachment of *L. monocytogenes* to the whole melon surfaces was significantly ($P < 0.05$) higher for sanitized melons. This phenomenon was most pronounced at the lowest inoculum level (10^3 CFU/mL).

Survival of *L. monocytogenes* on the Surface of Whole Melon and in Rind Homogenates

Populations of *L. monocytogenes* inoculated on sanitized and unsanitized whole melon surfaces gradually decreased during storage at 5C (Fig. 4). The decrease was more gradual on melons that had been sanitized with EtOH or chlorine before inoculation. Results for storage at 25C (data not shown) were similar to 5C storage. However, the decreases in populations of *L. monocytogenes* were more rapid on the surfaces of sanitized and unsanitized whole melons stored at 25C than at 5C.

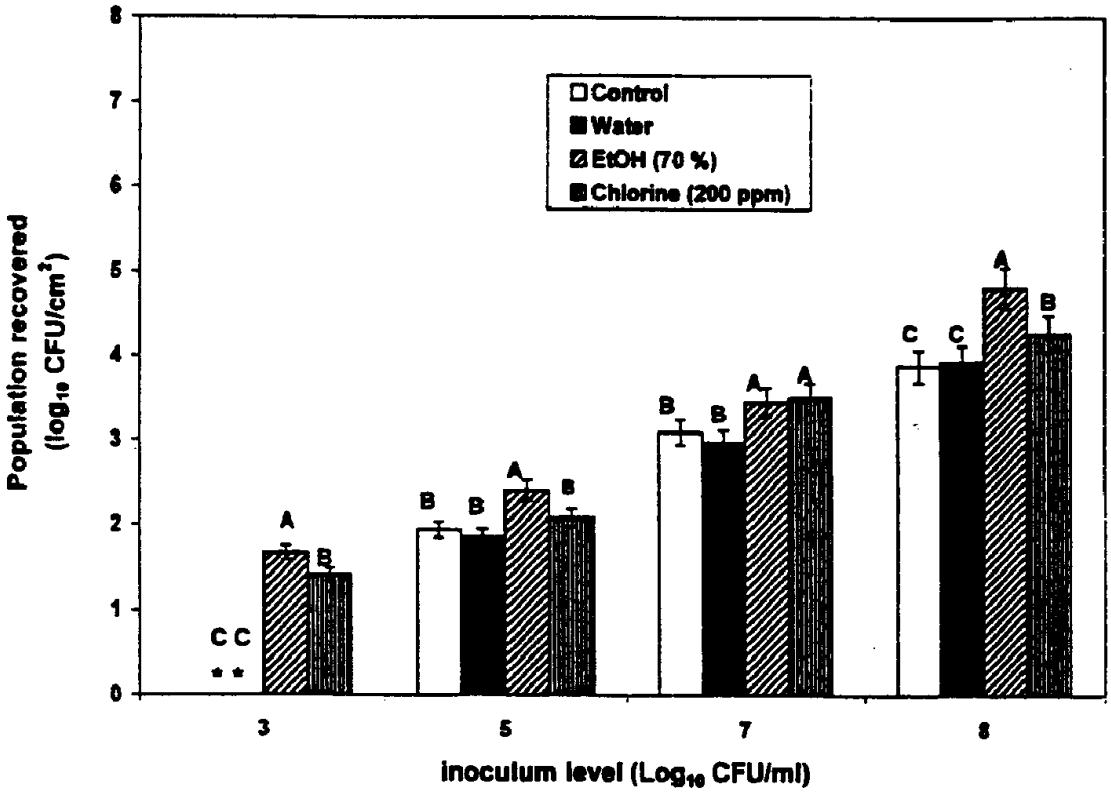

FIG. 3. EFFECT OF SANITIZER TREATMENTS AND INOCULUM LEVEL ON INITIAL ATTACHMENT OF *LISTERIA MONOCYTOGENES* ON WHOLE CANTALOUPE SURFACES

Error bars represent ± standard deviation. * = Below level of detection (6 cfu/cm²). Means of data for the same inoculum level not followed by the same letter are significantly different ($P < 0.05$).

Populations of *L. monocytogenes* (inoculated at 2.7 log CFU/mL) in nonsterile or sterile rind homogenates prepared from untreated whole melon incubated at 5, 10 and 25C are shown in Fig. 5. The population of *L. monocytogenes* in TSBY and sterile rind homogenates increased throughout the

incubation period at all three temperatures with more rapid growth at 25C. The population of *L. monocytogenes* in nonsterile rind homogenates exhibited no growth and declined to below detection at day 12 at 5C, day 9 at 10C and day 6 at 25C. Populations of *L. monocytogenes* (inoculated at 2.56 log CFU/mL) in sterile cantaloupe rind homogenates in the presence of added yeast and mold or lactic acid bacteria (inoculated at ~ 2.43 log CFU/mL) during incubation at 5C and 25C are shown in Fig. 6. Similar increases in the population of *L. monocytogenes* in sterile rind homogenates co-inoculated with lactic acid bacteria, aerobic mesophiles and *Pseudomonas* spp. were observed at 5C. At 25C, the presence of these classes of native microflora resulted in slower growth. In contrast, *Listeria monocytogenes* survived but showed little growth in sterile rind homogenates co-inoculated with yeast and mold at 5C and declined to below detection at day 6 with incubation at 25C.

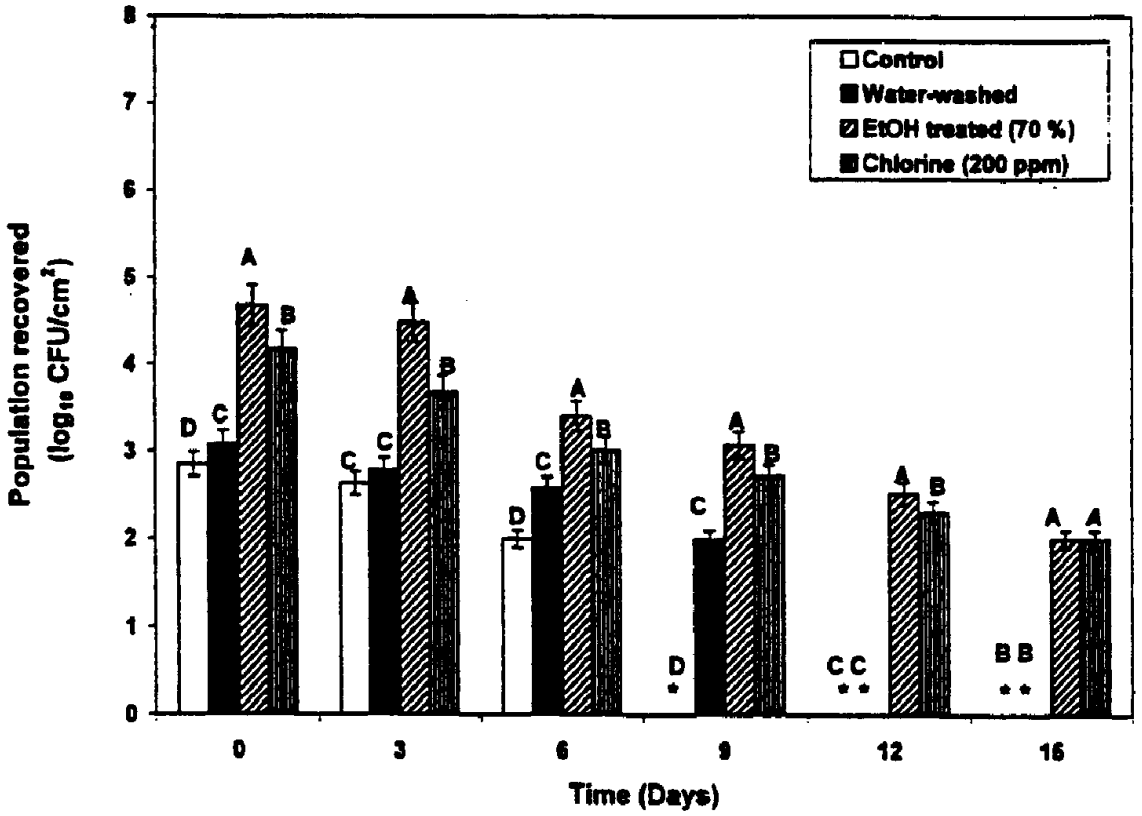

FIG. 4. SURVIVAL OF *LISTERIA MONOCYTOGENES* (INOCULUM LEVEL = 7.58 log_{10} CFU/ML) ON TREATED OR UNTREATED WHOLE CANTALOUPE SURFACES DURING STORAGE AT 5C

Error bars represent ± standard deviation. * = Below level of detection (6 cfu/cm^2). Means of data for the same day of storage not followed by the same letter are significantly different ($P < 0.05$).

EFFECT OF CANTALOUPE MICROFLORA ON *L. MONOCYTOGENES*

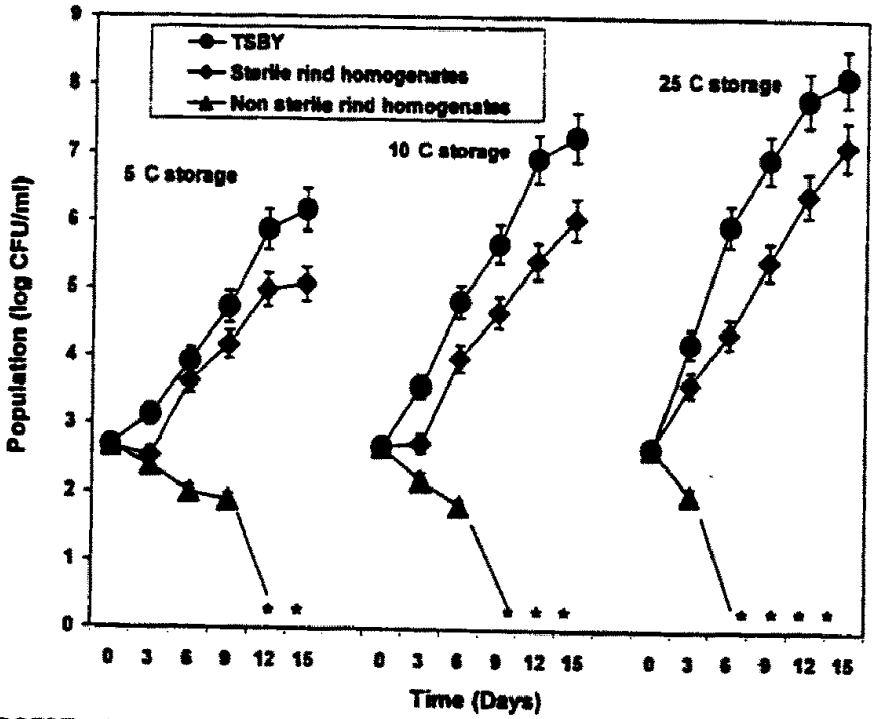

FIG. 5. POPULATION OF *L. MONOCYTOGENES* (INOCULATED AT 2.7 LOG CFU/ML) IN TSBY AND STERILE AND NONSTERILE RIND HOMOGENATES DURING STORAGE AT 5, 10 AND 25C FOR 15 DAYS

Error bars represent ± standard deviation. * = Below limit of detection (10 cfu/mL).

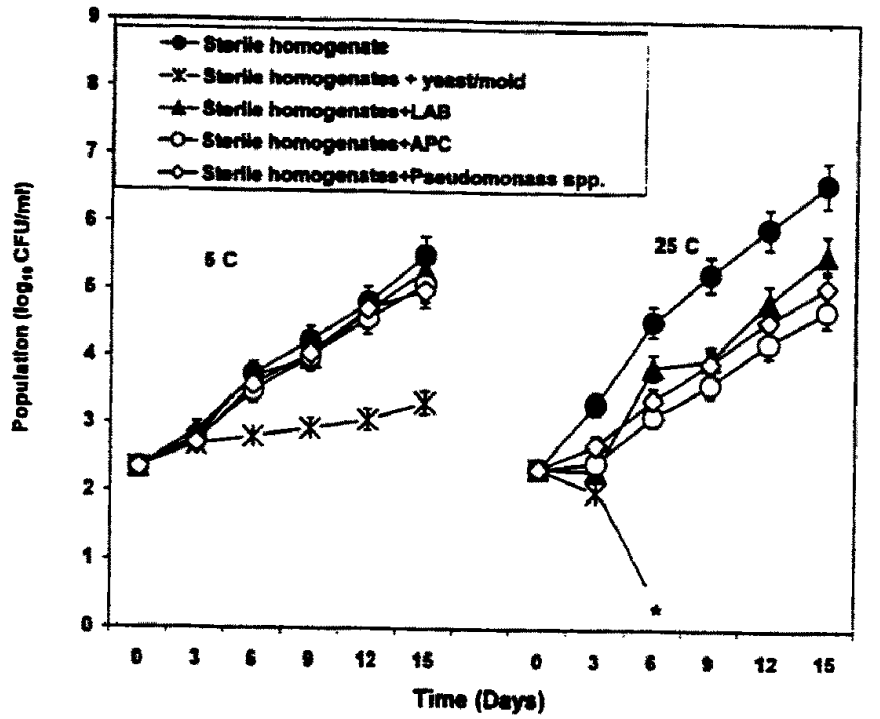

FIG. 6. GROWTH OF *L. MONOCYTOGENES* (INOCULATED AT 2.56 LOG CFU/ML) IN STERILE HOMOGENATES OF CANTALOUPE RIND WITH OR WITHOUT ADDED AEROBIC MESOPHILIC BACTERIA, *PSEUDOMONAS* SPP, YEAST AND MOLD OR LACTIC ACID BACTERIA (INOCULATED AT ~2.43 LOG CFU/ML) DURING INCUBATION AT 5C OR 25C

* = Below level of detection (6 cfu/mL). Error bars represent ± standard deviation.

Survival of *L. monocytogenes* on Fresh-Cut Pieces and In Fresh-Cut Homogenates

Populations of native microflora and *L. monocytogenes* on directly inoculated fresh-cut pieces prepared from untreated or treated whole melons with storage at an abusive temperature (10C) are shown in Fig. 7. The initial population of *L. monocytogenes* on the fresh-cut pieces was 2.6 log CFU/g. Aerobic mesophilic bacteria and yeast and mold outgrew *L. monocytogenes* on the fresh-cut pieces prepared from both treated and untreated melons. Populations of *L. monocytogenes* on fresh-cut pieces prepared from untreated melons increased slightly by day 4, but declined rapidly thereafter and could not be detected after day 10. Populations of *L. monocytogenes* on fresh-cut pieces prepared from EtOH treated whole melons slightly increased until day 4 followed by a gradual decline to a level below detection by day 15. Populations of *L. monocytogenes* in fresh-cut pieces prepared from chlorine treated whole melon increased up to day 6 and then declined, but the pathogen was still detected in fresh-cut pieces at day 15.

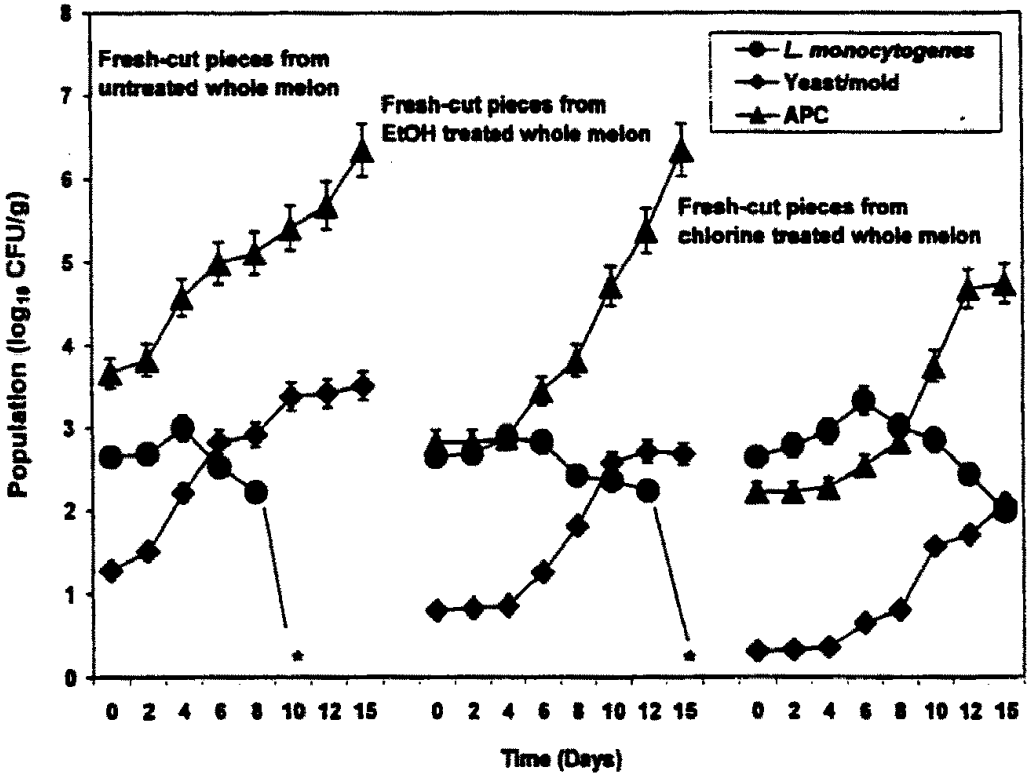

FIG. 7. POPULATION OF *LISTERIA MONOCYTOGENES*, AEROBIC MESOPHILES AND YEAST AND MOLD ON FRESH-CUT PIECES PREPARED FROM TREATED OR UNTREATED WHOLE MELONS AND DIRECTLY INOCULATED WITH *L. MONOCYTOGENES* (2.56 LOG CFU/g), DURING STORAGE AT 10C FOR 15 DAYS
* = Below limit of detection (7 cfu/g). Error bars represent ± standard deviation.

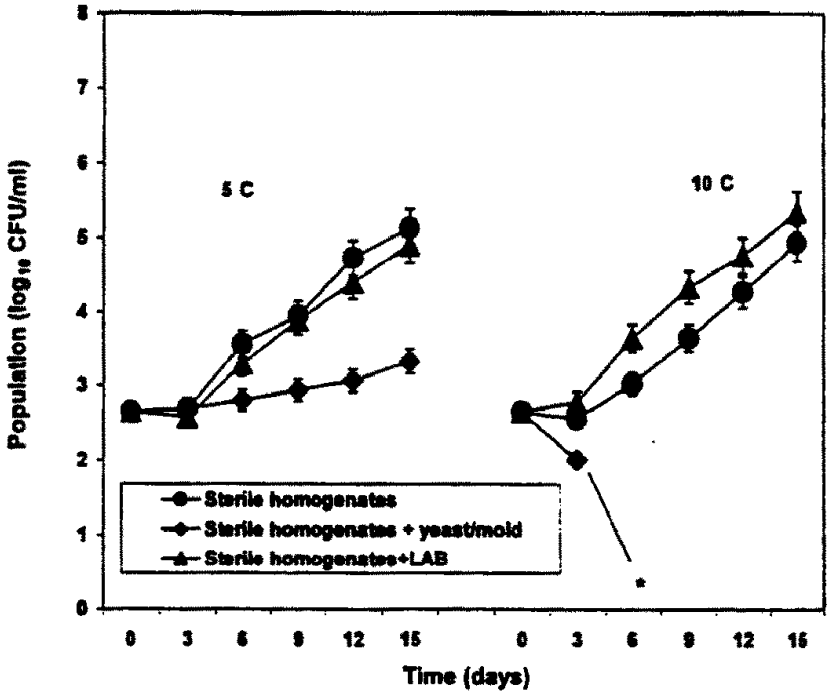

FIG. 8. POPULATION OF *LISTERIA MONOCYTOGENES* (INOCULATED AT 2.56 LOG CFU/ML) IN FRESH-CUT CANTALOUPE HOMOGENATES WITH ADDED LACTIC ACID BACTERIA OR YEAST AND MOLD (2.43 CFU/ML) DURING INCUBATION AT 5 AND 10C

* = Below limit of detection (6 cfu/mL). Error bars represent ± standard deviation.

The effect of addition of lactic acid bacteria and yeast and mold (at ~ 2.43 log_{10} CFU/mL each) to sterile fresh-cut homogenates inoculated with *L. monocytogenes* (2.35 log CFU/mL) incubated at 5C and at an abusive temperature (10C) is shown in Fig. 8. Growth of *Listeria monocytogenes* was not inhibited throughout incubation in the presence of lactic acid bacteria at either temperature. In contrast, the presence of yeast and mold reduced the growth rate at 5C. At 10C the presence of yeast and mold resulted in a sharp decline of *L. monocytogenes* with levels below detection by day 6.

DISCUSSION

The ability of washes with EtOH (70%) and chlorine (200 ppm) and the inability of water washes to reduce the native microflora on the surfaces of whole cantaloupes confirm the results of our earlier studies (Ukuku *et al.* 2001; Ukuku and Fett 2002a). Also confirmed by this study were previous data

(Ukuku and Fett 2002a) indicating that sanitizing whole melons with chlorine (200 ppm) leads to a reduction of native microflora on fresh-cut pieces. Attachment of *L. monocytogenes* to cantaloupe rind increased when the population of naturally occurring microflora was reduced by the sanitizer treatments. We previously reported similar results for attachment of *E. coli* to whole melons treated with EtOH (70%) (Ukuku *et al.* 2001). However, it is not clear if the increases in attachment are due to the presence of a finite number of microbial binding sites, some of which are made available by the sanitizer treatments, or to chemical and/or physical changes in the melon surface that are imparted by the treatments.

There have been several reports of antagonism of *L. monocytogenes* by microorganisms native to plant surfaces. Using an *in vitro* surface model system, Francis and O'Beirne (1998a) demonstrated that the lettuce isolates *Enterobacter cloaceae* and *Enterobacter agglomerans* inhibited growth of *L. monocytogenes* in ambient air. They also reported antagonism by the two *Enterobacter* spp. against *L. innocua* in a lettuce liquid medium model system (Francis and O'Beirne 1998b). A complex bacterial population recovered from endive leaves completely inhibited the growth of *L. monocytogenes* in an endive leaf extract liquid medium (Carlin *et al.* 1996). Enhanced survival and growth of *L. monocytogenes* on the surface of leafy vegetables was reported by several researchers after surface treatments with antimicrobial agents (Beņnik *et al.* 1996; Carlin *et al.* 1996; Francis and O'Beirne 1997).

Our data indicate that the native microflora on whole melons and fresh-cut pieces are also antagonistic towards growth and survival of *L. monocytogenes*. Reducing the native microflora on the surface of whole melons led to greater survival of the pathogen during storage at 5 and 25C although the pathogen did not multiply. The inability of *L. monocytogenes* to grow on the surface of untreated whole cantaloupe melon confirms the results of our earlier study (Ukuku and Fett 2002b). Water washes only reduced the level of yeast and mold, but this reduction led to increased survival of the pathogen, suggesting that the native yeast and mold population is antagonistic. Increased survival of *L. monocytogenes* was noted after sanitizing with either EtOH (70%) or chlorine (200 ppm); treatments that led to reductions in all five classes of native microflora. This increase in survival after sanitizing may have been due to further reduction in populations of yeast and mold and/or to reduced populations of other antagonistic classes of native microflora. The use of more effective sanitizer treatments leading to greater reductions of the native microflora may allow for growth of *L. monocytogenes* on the whole melon surface.

The antagonistic nature of the native microflora on whole melon surfaces was also supported by the results of the *in vitro* assays in rind homogenates. *Listeria monocytogenes* grew only in sterile rind homogenates. In nonsterile homogenates the presence of the native microflora led to a rapid decline in

pathogen populations at both incubation temperatures. Addition of yeast and mold isolated from whole melon surfaces to sterile rind homogenates strongly inhibited growth of the pathogen during incubation at 5C and led to a rapid decline in populations during incubation at 25C further indicating that native yeast and mold are significant antagonists of the pathogen. Lactic acid bacteria isolated from whole melons were not antagonistic to the pathogen even though as a group their ability to produce bacteriocins and other metabolites inhibitory towards *L. monocytogenes* is well known (Briedt and Fleming 1997; Vescovo *et al.* 1996).

Our data also demonstrated the antagonism of native microflora towards *L. monocytogenes* on fresh-cut pieces. A reduction of the native microflora on fresh-cut pieces prior to inoculation due to sanitizing whole cantaloupe before cutting led to increased growth and survival of the pathogen during storage of the inoculated pieces at 10C. No *L. monocytogenes* could be detected on fresh-cut pieces prepared from untreated whole melon by day 10 of storage. In an earlier study the pathogen also could not be detected by day 10 of storage at 4C when fresh-cut pieces were prepared from control or water-washed whole melons inoculated with *L. monocytogenes* (Ukuku and Fett 2002b). These previous results may also have been due to the presence of antagonistic native microflora. As was the case for sterile rind homogenates, addition of native yeast and mold, but not lactic acid bacteria, led to reduced growth and survival of the pathogen during incubation at 5 or 10C.

Application of chemical or physical antimicrobial interventions to whole melons before shipment or fresh-cut preparation may be desirable due to reduction in transfer of bacterial human pathogens such as *L. monocytogenes* from the rind to the inner flesh during cutting operations. However, as indicated by the results of this study, the recontamination of whole melons or fresh-cut pieces with *L. monocytogenes* after sanitizing melons is a food safety concern. Such recontamination might result from poor plant sanitation or use of improper handling or packaging procedures after the melons were sanitized.

On the other hand, addition of a biocontrol agent(s) to inhibit survival and growth of *L. monocytogenes* on whole melons or on fresh-cut pieces might be a viable control strategy. Native yeast and mold, either as complex communities or individual isolates, may be good candidates for use as biological control agents if their presence does not negatively impact on product quality. A commercial product containing the yeast *Candida oleophila* is currently available for control of postharvest fungal rots of fruits, indicating the feasibility of this approach (El-Ghaouth *et al.* 2000). Biological control agents may be even more effective against *L. monocytogenes* when used in combination with antimicrobial chemicals (Leverentz *et al.* 2003).

In conclusion, *L. monocytogenes* attached in greater numbers to cantaloupe rinds when populations of naturally occurring microflora were reduced. Also,

survival of inoculated *L. monocytogenes* during storage of sanitized melons or fresh-cut pieces prepared from sanitized whole melons was greater compared to that for unsanitized melons. Growth and survival of *L. monocytogenes* in sterile rind homogenates was significantly reduced in the presence of added native yeast and mold. We conclude that certain classes of native microflora on the surface of whole and fresh-cut cantaloupe are antagonistic to the survival and growth of *L. monocytogenes* during storage. Recontamination of whole or fresh-cut melon, having a reduced level of native microflora, with *L. monocytogenes* is a food safety concern.

ACKNOWLEDGMENTS

The authors acknowledge the valuable assistance of Dr. John G. Phillips in statistical analysis of the data, Ms. Manisha Amin and Mr. Larry Revear for excellent technical support and Dr. Larry Beuchat for supplying bacterial strains.

REFERENCES

BENNIK, M.H.J., PEPPELENBOS, H.W., NGUYEN-THE, C., CARLIN, F., SMID, E.J. and GORRIS, L.G.M. 1996. Microbiology of minimally processed, modified-atmosphere packaged chicory endive. Postharvest Biol. Technol. *9*, 209–221.

BERRANG, M.E., BRACKETT, R.E. and BEUCHAT, L.R. 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under a controlled atmosphere. J. Food Prot. *52*, 702–705.

BEUCHAT, L.R. 1996. *Listeria monocytogenes*: incidence on vegetables. Food Control *7*, 223-228.

BEUCHAT, R.L. and BRACKETT, R.E. 1991. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. Appl. Environ. Microbiol. *57*, 1367–1371.

BRACKETT, R.E. 1988. Presence and persistence of *Listeria monocytogenes* in food and water. Food Technol. *42*, 162–164.

BREIDT, F. and FLEMING, H.P. 1997. Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. Food Technol. *51*, 44–49.

CARLIN, F. and NGUYEN-THE, C. 1994. Fate of *Listeria monocytogenes* on four types of minimally processed green salads. Lett. Appl. Microbiol. *18*, 222–226.

CARLIN, F., NGUYEN-THE, C. and MORRIS, C. 1996. Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive. J. Food Prot. *59*, 698–703.

COX, L.J. *et al.* 1989. *Listeria* spp. in food processing, non-food and domestic environments. Food Microbiol. *6*, 49–61.

EL-GHAOUTH, A., SMILANICK, J.L., WISNIEWSKI, M. and WILSON, C.L. 2000. Improved control of apple and citrus fruit decay with a combination of *Candida saitoana* and 2-deoxy-D-glucose. Plant Dis. *84*, 249–253.

FARBER, J.M. and PETERKIN, P.I. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. *55*, 476–511.

FARBER, J.M., SANDERS, G.W. and JOHNSTON, M.A. 1989. A survey of various foods for the presence of *Listeria* species. J. Food Prot. *52*, 456–458.

FDA. 2003. Duck Delivery Produce Recalls cut honeydew and cut cantaloupe melon for possible health risk. http://www.safetyalerts.com/recall/f/02.2/f0002828.htm

FRANCIS, G.A. and O'BEIRNE, D. 1997. Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. Intern. J. Food. Sci. Technol. *32*, 141–151.

FRANCIS, G.A. and O'BEIRNE, D. 1998a. Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using surface model system. Intern. J. Food Sci. Technol. *33*, 465–476.

FRANCIS, G.A. and O'BEIRNE, D. 1998b. Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. Intern. J. Food. Sci. Technol. *33*, 477–488.

HEISICK, J.E., ROSAS-MARTY, L. and TATINI, S.R. 1995. Enumeration of viable *Listeria* species and *Listeria monocytogenes* in foods. J. Food Prot. *58*, 733–736.

HEISICK, J.E., WAGNER, D.E., NIERMAN, M.L. and PEELER, J.T. 1989. *Listeria* spp. found on fresh market produce. Appl. Environ. Microbiol. *55*, 1925–1927.

KROGER, C. 2000. Salad factory issues limited recall of salads. The Packer. CVII, *52*, p. A3.

LEIBINGER, W., BREUKER, B., HAHN, M. and MENDGEN, K. 1997. Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. Phytopathology *87*, 1103–1110.

LEVERENTZ, B. *et al.* 2003. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and bacteriocin. Appl. Environ. Microbiol. *69*, 4519–4526.

LOVETT, J. and HITCHINS, A.D. 1988. *Listeria* isolation. Bacteriological Analytical Manual (BAM), FDA, Chapter 29, (Revised October 13, 1988). Supplement to 6th Ed., Assoc. Offic. Anal. Chem. Arlington, VA.

MAFU, A.A., ROY, D., GOULET, J. and MAGNY, P. 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene and rubber surfaces after short contact time. J. Food Prot. *53*, 742–746.

MARSTON, E.V. 1995. Fresh-cut fruits: maximizing quality. Cutting Edge *9*, 3–5.

MILLER JR., R.G. 1981. *Simultaneous Statistical Inference*, 2nd Ed., pp. 67–70, Springer-Verlag, New York.

NORRIS, J.R. and RIBBONS, D.W. 1971. *Methods in Microbiology*, Vol. 4, (C. Booth, ed.) Academic Press, London.

REUTER, G. 1985. Elective and selective media for lactic acid bacteria. Intern. J. Food Microbiol. 2, 55–68.

TORRIANI, S., ORSI, C. and VESCOVO, M. 1997. Potential of *Lactobacillus casei*, culture permeate, and lactic acid to control microorganisms in ready-to-use vegetables. J. Food Prot. *60*, 1564–1567.

UKUKU, D.O. and FETT, W.F. 2002a. Effectiveness of chlorine and nisin-EDTA treatments of whole melons and fresh-cut pieces for reducing native microflora and extending shelf-life. J. Food Safety *22*, 231–253.

UKUKU, D.O. and FETT, W.F. 2002b. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. J. Food Prot. *65*, 924–930.

UKUKU, D.O., PILIZOTA, V. and SAPERS, G.M. 2001. Influence of washing treatments on native microflora and *Escherichia coli* population of inoculated cantaloupes. J. Food Safety *21*, 31-47.

UKUKU, D.O. and SAPERS, G.M. 2001. Effect of sanitizer treatments on *Salmonella stanley* attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Prot. *64*, 1286–1291.

VESCOVO, M., TORRIANI, S., ORSI, C., MACCHIAROLO, F. and SCOLARI, G. 1996. Application of antimicrobial-producing lactic acid bacteria to control pathogens in ready-to-use vegetables. J. Appl. Bacteriol. *81*, 113–119.

WONG, S., STREET, D., DELGADO, S.I. and KLONTZ, K.C. 2000. Recalls of foods and cosmetics due to microbial contamination reported to the U.S. Food and Drug Administration. J. Food Prot. *63*, 1113–1116.